# abcam

### Product datasheet

## Anti-Prion protein PrP antibody [EP1802Y] ab52604

Recombinant RabMAb

#### \*\*\*\*\* 2 Abreviews 31 References 9 Images

#### Overview

Product name	Anti-Prion protein PrP antibody [EP1802Y]
Description	Rabbit monoclonal [EP1802Y] to Prion protein PrP
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt (Intra), WB, IHC-P Unsuitable for: ICC
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human, rat and mouse brain tissue lysates. SK-MEL-28 and Neuro-2a whole cell lysates. <u>ab74056</u> ; IHC-P: brain glioma tissue, Human, mouse and rat cerebrum tissue; Flow Cyt (intra): SH-SY5Y cells.
General notes	<ul> <li>This product is a recombinant monoclonal antibody, which offers several advantages including:</li> <li>High batch-to-batch consistency and reproducibility</li> <li>Improved sensitivity and specificity</li> <li>Long-term security of supply</li> <li>Animal-free production</li> <li>For more information <u>see here</u>.</li> <li>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb<sup>®</sup> patents</u>.</li> </ul>

Properties	
Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP1802Y

#### Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab52604 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/70. For unpurified use at 1/00. <u><b>ab172730</b></u> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★	1/5000 - 1/10000. Detects a band of approximately 28 kDa (predicted molecular weight: 28 kDa).
IHC-P		1/500. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. See <b>IHC antigen retrieval protocols</b> .

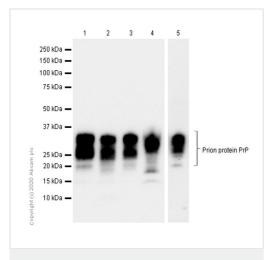
**Application notes** 

Is unsuitable for ICC.

Target	
Function	The function of PrP is still under debate. May play a role in neuronal development and synaptic plasticity. May be required for neuronal myelin sheath maintenance. May play a role in iron uptake and iron homeostasis (By similarity). Isoform 2 may act as a growth suppressor by arresting the cell cycle at the G0/G1 phase. Soluble oligomers are toxic to cultured neuroblastoma cells and induce apoptosis (in vitro).
Involvement in disease	<ul> <li>Note=PrP is found in high quantity in the brain of humans and animals infected with neurodegenerative diseases known as transmissible spongiform encephalopathies or prion diseases, like: Creutzfeldt-Jakob disease (CJD), fatal familial insomnia (FFI), Gerstmann-Straussler disease (GSD), Huntington disease-like type 1 (HDL1) and kuru in humans; scrapie in sheep and goat; bovine spongiform encephalopathy (BSE) in cattle; transmissible mink encephalopathy (TME); chronic wasting disease (CWD) of mule deer and elk; feline spongiform encephalopathy (BSE) in cattle; transmissible mink encephalopathy (FSE) in cats and exotic ungulate encephalopathy (EUE) in nyala and greater kudu. The prion diseases illustrate three manifestations of CNS degeneration: (1) infectious (2) sporadic and (3) dominantly inherited forms. TME, CWD, BSE, FSE, EUE are all thought to occur after consumption of prion-infected foodstuffs.</li> <li>Defects in PRNP are the cause of Creutzfeldt-Jakob disease (CJD) [MIM:123400]. CJD occurs primarily as a sporadic disorder (1 per million), while 10-15% are familial. Accidental transmission of CJD to humans appears to be iatrogenic (contaminated human growth hormone (HGH), corneal transplantation, electroencephalographic electrode implantation, etc.).</li> <li>Epidemiologic studies have failed to implicate the ingestion of infected annimal meat in the pathogenesis of CJD in human. The triad of microscopic features that characterize the prion diseases consists of (1) spongiform degeneration of neurons, (2) severe astrocytic gliosis that often appears to be out of proportion to the degree of nerve cell loss, and (3) amyloid plaque formation. CJD is characterized by progressive dementia and myoclonic seizures, affecting adult in mid-life. Some patients present sleep disorders, abnormalities of high cortical function, cerebellar and corticospinal disturbances. The disease ends in death after a 3-12 months illness.</li> </ul>

Somuonoo oimilaritioo	Defects in PRNP are the cause of fatal familial insomnia (FFI) [MIM:600072]. FFI is an autosomal dominant disorder and is characterized by neuronal degeneration limited to selected thalamic nuclei and progressive insomnia. Defects in PRNP are the cause of Gerstmann-Straussler disease (GSD) [MIM:137440]. GSD is a heterogeneous disorder and was defined as a spinocerebellar ataxia with dementia and plaquelike deposits. GSD incidence is less than 2 per 100 million live births. Defects in PRNP are the cause of Huntington disease-like type 1 (HDL1) [MIM:603218]. HDL1 is an autosomal dominant, early onset neurodegenerative disorder with prominent psychiatric features. Defects in PRNP are the cause of kuru (KURU) [MIM:245300]. Kuru is transmitted during ritualistic cannibalism, among natives of the New Guinea highlands. Patients exhibit various movement disorders like cerebellar abnormalities, rigidity of the limbs, and clonus. Emotional lability is present, and dementia is conspicuously absent. Death usually occurs from 3 to 12 month after onset. Defects in PRNP are the cause of spongiform encephalopathy with neuropsychiatric features (SENF) [MIM:606688]; an autosomal dominant presenile dementia with a rapidly progressive and protracted clinical course. The dementia was characterized clinically by frontotemporal features, including early personality changes. Some patients had memory loss, several showed aggressiveness, hyperorality and verbal stereotypy, others had parkinsonian symptoms.
Sequence similarities	Belongs to the prion family.
Domain	The normal, monomeric form has a mainly alpha-helical structure. The disease-associated, protease-resistant form forms amyloid fibrils containing a cross-beta spine, formed by a steric zipper of superposed beta-strands. Disease mutations may favor intermolecular contacts via short beta strands, and may thereby trigger oligomerization. Contains an N-terminal region composed of octamer repeats. At low copper concentrations, the sidechains of His residues from three or four repeats contribute to the binding of a single copper ion. Alternatively, a copper ion can be bound by interaction with the sidechain and backbone amide nitrogen of a single His residue. The observed copper binding stoichiometry suggests that two repeat regions cooperate to stabilize the binding of a single copper concentrations, each octamer can bind one copper ion by interactions with the His sidechain and Gly backbone atoms. A mixture of binding types may occur, especially in the case of octamer repeat expansion. Copper binding may stabilize the conformation of this region and may promote oligomerization.
Post-translational modifications	The glycosylation pattern (the amount of mono-, di- and non-glycosylated forms or glycoforms) seems to differ in normal and CJD prion. Isoform 2 is sumoylated by SUMO1.
Cellular localization	Cell membrane. Golgi apparatus and Cytoplasm. Nucleus. Accumulates outside the secretory route in the cytoplasm, from where it relocates to the nucleus.

Images



Western blot - Anti-Prion protein PrP antibody [EP1802Y] (ab52604)

**All lanes :** Anti-Prion protein PrP antibody [EP1802Y] (ab52604) at 1/5000 dilution (Purified)

Lane 1 : Human brain lysate

Lane 2 : Mouse brain lysate

Lane 3 : Rat brain lysate

Lane 4 : SK-MEL-28 (Human malignant melanoma ) whole cell lysate

Lane 5 : Neuro-2a (Mouse neuroblastoma neuroblast) whole cell lysate

Lysates/proteins at 20 µg per lane.

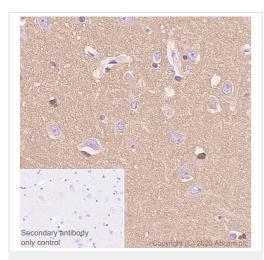
#### Secondary

**All lanes :** Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Predicted band size: 28 kDa Observed band size: 20-37 kDa

The molecular weights observed represent different glycosation states and are consistent with what has been described in the literature (PMID: 20670940, PMID: 19568430, PMID: 15240877 and PMID: 22558368).

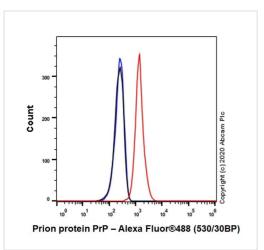
Blocking/Diluting buffer: 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Prion protein PrP antibody [EP1802Y] (ab52604)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cerebrum tissue sections labeling Prion protein PrP with purified ab52604 at 1/500 dilution (1.32 µg/mL). Heat mediated antigen retrieval was performed using Bond™ Epitope Retrieval Solution 1 (pH 6.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain. The immunostaining was performed on a Leica Biosystems

BOND® RX instrument.



Flow Cytometry (Intracellular) - Anti-Prion protein PrP antibody [EP1802Y] (ab52604) Intracellular Flow Cytometry analysis of SH-SY5Y (Human neuroblastoma epithelial cell) cells, labeling Prion protein PrP with Purified ab52604 at 1/70 dilution (10µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluorr<sup>®</sup> 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Western blot - Anti-Prion protein PrP antibody [EP1802Y] (ab52604)

Anti-Prion protein PrP antibody [EP1802Y] (ab52604) at 1/5000 dilution (unpurified) + Recombinant Mouse Prion protein PrP (<u>ab74056</u>) at 0.01 μg

#### Secondary

Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (<u>ab97080</u>) at 1/5000 dilution

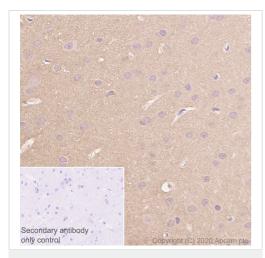
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 28 kDa

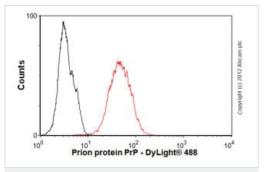
#### Exposure time: 10 seconds

This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% Bovine Serum Albumin before being incubated with ab52604 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution **ab133406**.

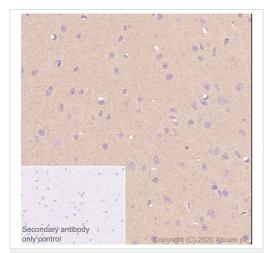


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Prion protein PrP antibody [EP1802Y] (ab52604) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat cerebrum tissue sections labeling Prion protein PrP with purified ab52604 at 1/500 dilution (1.32 µg/mL). Heat mediated antigen retrieval was performed using Bond<sup>™</sup> Epitope Retrieval Solution 1 (pH 6.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain. The immunostaining was performed on a Leica Biosystems

BOND® RX instrument.



Flow Cytometry (Intracellular) - Anti-Prion protein PrP antibody [EP1802Y] (ab52604) Overlay histogram showing SH-SY5Y cells stained with unpurifiedab52604 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific proteinprotein interactions followed by the antibody (ab52604, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10<sup>6</sup> cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in SH-SY5Y cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Prion protein PrP antibody [EP1802Y] (ab52604)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse cerebrum tissue sections labeling Prion protein PrP with purified ab52604 at 1/500 dilution (1.32 µg/mL). Heat mediated antigen retrieval was performed using Bond™ Epitope Retrieval Solution 1 (pH 6.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Prion protein PrP antibody [EP1802Y] (ab52604)



Immunohistochemical analysis of brain glioma using ab52604 (unpurified) at a dilution of 1/100. Heat mediated antigen retrieval was performed with citrate buffer pH 6 before commencing with IHC staining protocol.

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